ICES Journal of Marine Science Advance Access published January 10, 2016

ICES Journal of Marine Science

ICES Journal of Marine Science; doi:10.1093/icesjms/fsv258

End of the century CO_2 concentrations do not have a negative effect on vital rates of *Calanus finmarchicus*, an ecologically critical planktonic species in North Atlantic ecosystems

Jeffrey A. Runge[1]*, David M. Fields[2], Cameron R. S. Thompson[2], Steven D. Shema[3], Reidun M. Bjelland[3], Caroline M. F. Durif[3], Anne Berit Skiftesvik[3], and Howard I. Browman[3]

[1]*School of Marine Sciences, University of Maine and Gulf of Maine Research Institute, 350 Commercial Street, Portland, ME 04103, USA*
[2]*Bigelow Laboratory for Ocean Sciences, East Boothbay, ME 04544, USA*
[3]*Institute of Marine Research, Austevoll Research Station, Storebø 5392, Norway*

**Corresponding author: tel: +1 207 228 1652; fax: +1 207 773 8672; e-mail: jeffrey.runge@maine.edu*

Runge, J. A., Fields, D. M., Thompson, C. R. S., Shema, S. D., Bjelland, R. M., Durif, C. M. F., Skiftesvik, A. B., and Browman, H. I. End of the century CO_2 concentrations do not have a negative effect on vital rates of *Calanus finmarchicus*, an ecologically critical planktonic species in North Atlantic ecosystems. – ICES Journal of Marine Science, doi: 10.1093/icesjms/fsv258.

Received 1 July 2015; revised 4 December 2015; accepted 7 December 2015.

The Subarctic copepod, *Calanus finmarchicus*, is an ecologically critical foundation species throughout the North Atlantic Ocean. Any change in the abundance and distribution of *C. finmarchicus* would have profound effects on North Atlantic pelagic ecosystems and the services that they support, particularly on the coastal shelves located at the southern margins of the species' range. We tested the hypothesis that the physiological rates and processes of *C. finmarchicus*, determining its vital rates, are unaffected by increases in CO_2 concentration predicted to occur in the surface waters of the ocean during the next 100 years. We reared *C. finmarchicus* from eggs to adults at a control (580 μatm, the ambient concentration at the laboratory's seawater intake) and at predicted mid-range (1200 μatm) and high (1900 μatm) pCO_2. There was no significant effect of pCO_2 on development times, lipid accumulation, feeding rate, or metabolic rate. Small but significant treatment effects were found in body length and mass (in terms of dry, carbon and nitrogen mass), notably a somewhat larger body size at the mid-pCO_2 treatment; that is, a putatively beneficial effect. Based on these results, and a review of other studies of *Calanus*, we conclude that the present parameterizations of vital rates in models of *C. finmarchicus* population dynamics, used to generate scenarios of abundance and distribution of this species under future conditions, do not require an "ocean acidification effect" adjustment. A review of research on planktonic copepods indicates that, with only a few exceptions, impacts of increased CO_2 are small at the levels predicted to occur during the next century.

Keywords: climate change, copepods, grazing rate, growth and development rate, negative result, non-effect, Ocean acidification, respiration rate.

Introduction

Predictions of long-term change in pCO_2 and pH in the ocean, and consequences for marine life, has driven intense research activity into the effects of these drivers on marine organisms (e.g. Caldeira and Wickett, 2003; Fabry *et al.*, 2008; Dupont and Pörtner, 2013). Meta-analyses indicate variable responses to pCO_2 among taxa, species within taxa, populations within species, and individuals in any given experiment (e.g. Kroeker *et al.*, 2010; Whiteley, 2011; Garrard *et al.*, 2013; Wittmann and Portner, 2013). While this rapidly growing field of research indicates that some components of marine foodwebs may be vulnerable to ocean acidification, these differences in sensitivity make it difficult to predict the extent to which future change will disrupt ecosystems (e.g. Dupont *et al.*, 2010; Gaylord *et al.*, 2011).

In pelagic ecosystems of the northern North Atlantic Ocean, the Subarctic planktonic copepod, *Calanus finmarchicus*, is an ecologically critical foundation species (Melle *et al.*, 2014; Runge *et al.*, 2015). The lipid-rich stages serve as a primary source of energy to consumers such as herring (*Clupea harengus*), mackerel (*Scomber scombrus*), capelin (*Mallotus villosus*), and sand lance (*Ammodytes*

spp.) that are in turn prey for top predators, including groundfish, tuna (*Thunnus thynnus*), marine mammals, and many seabirds (e.g. Skjoldal, 2004; Baumgartner *et al.*, 2007; Johnson *et al.*, 2011; Trenkel *et al.*, 2014). Since *C. finmarchicus* plays such a crucial role, any impacts of increased pCO_2 would likely have important consequences for coastal and shelf ecosystems of the northern North Atlantic.

Approaches developed to understand and predict population responses of *C. finmarchicus* and other zooplankton species to environmental change involve life cycle modelling (e.g. Carlotti *et al.*, 2000; Maps *et al.*, 2012). Over the past several decades, advances in modelling have made it possible to couple these life cycle models with physical models that simulate water temperature and advective transport, allowing analysis of both the biological and physical processes determining the species' population dynamics (e.g. Speirs *et al.*, 2006; Maps *et al.*, 2011; Hjollo *et al.*, 2012; Curchitser *et al.*, 2013). Decades of research have provided quantitative knowledge of the *C. finmarchicus* physiological rates that are needed to parameterize life cycle models (Melle *et al.*, 2014). These models would have to be re-parameterized if there are significant impacts of increased oceanic pCO_2 on these rates.

In this study, we investigated growth, development, feeding, and respiration rates of *C. finmarchicus* in response to increased dissolved CO_2. We synthesized these findings with results from other studies on *Calanus* spp. to assess the impact of increased pCO_2 levels (predicted by realistic global change scenarios) on the physiological rates and processes in *C. finmarchicus* that determine its vital rates. We also compared the responses of *C. finmarchicus* with reported responses of other species in the genus, and across the Copepoda, in an effort to identify general patterns and mechanisms underlying their responses to future increases in pCO_2.

Material and methods

Experimental design

Eggs were collected from wild-caught females and reared through all life stages in three replicate tanks for each of a control and two pCO_2 treatment levels. Two of the replicate tanks were monitored frequently, as described below, to measure developmental rates, body size, carbon, nitrogen and dry mass of each stage, and lipid accumulation at stage C5, as well as respiration and feeding rates at selected stages. The third replicate tank for each treatment was held under the same experimental conditions and served as a reserve if the two other replicates did not provide a sufficient quantity of samples as the experiment progressed.

Facilities and analysis of carbonate chemistry variables

Experiments were conducted at the Austevoll Research Station, Institute of Marine Research, Norway (60.086 N, 5.262 E). Ambient seawater (serving as the control) was pumped from the Bjørn afjord at a depth of 160 m to the laboratory facilities, where it was first sand filtered then passed through a 20 μm Arcal disc filter before entering the experimental system. The ambient pH ($_{NBS}$) of seawater at the intake depth is ∼7.95, corresponding to a pCO_2 of ∼580 μatm. A depth of 160 m represents the lower end of vertical range of *C. finmarchicus* stages C1–C4 in spring in the Northeast Atlantic and is shallower than overwintering depths of stage C5 (e.g. Williams and Conway, 1988). Water temperature flowing into the preparation room next to the experimental facilities was about 12°C, rising slowly during the experimental period. In the preparation room, a high pCO_2 stock seawater solution was maintained at a pH of ∼5.8. The stock solution was mixed into holding tanks (100 l) to create seawater at treatment pCO_2 levels (ambient control, 1200 and 1900 μatm: Table 1). The pH in each tank was maintained at pre-set pH levels by adding stock solution to the tank using dosage pumps (IWAKI) controlled by feedback from pH electrodes/controllers (Endress and Hauser, Liquiline CM 442). The pCO_2 treatment water in the holding tanks was then pumped to three header tanks in the temperature-controlled (12°C) experiment room and distributed by gravity to the experimental tanks. The water level in the holding tanks was maintained by flotation valves. To maintain stable temperature and pH, water flow into the header tanks was much higher than the flow from header tanks to the experimental tanks. The 40 l experimental tanks, identical for all treatments, were 44 cm in diameter and made of high-density polyethylene (HDPE). The inlet tube into each experimental tank was fitted with a 60 μm mesh nitex screen, cleaned periodically to prevent contamination of the tank by microplankton from the plumbing system. The flow rate of water into the experimental tanks varied between 5 and 12 l h^{-1}. Water in the experimental tanks drained through a screen (70 μm) placed over a perforated 35 mm diameter plastic standpipe running the height of the tank. This setup ensured very low exit flow rates at any point along the tube. As the copepods grew during the experiment, the size of the exit screen was increased to 150 μm. Early life stage copepods in close vicinity of the exit pipe could easily swim away from the suction and were not drawn back in, with no indication of stress or abnormal behavior. This system provided a sufficiently gentle environment for rearing copepod life stages while maintaining high water replacement within the tanks and stable pCO_2 treatment levels.

Routine measurement of tank conditions and analysis of carbonate chemistry were conducted to assess consistency of conditions during the experiment. Temperature and salinity in the tanks were measured daily with a hand-held multimeter (Cond 340i conductivity meter: WTW, Germany). The pH level in each exposure tank was measured daily in a 100 ml sample using a Mettler-Toledo pH meter equipped with a Mettler-Toledo InLab® ExpertPro pH-probe, calibrated with 4.00, 7.00, and 9.00 buffers (Certipur® buffer solutions, Merck KGaA, 64271 Darmstadt, Germany, traceable to standard reference material from NIST (NBS) (Andersen *et al.*, 2013). The daily electrode pH (mV) was corrected to the spectrophotometrically determined pH (pH_{tot}; see below) by plotting the mV from the pH electrode as a function of the (pH_{spec}). Over the course of the experiment electrode voltage was highly correlated with pH_{spec} measurements ($r^2 = 0.985$; Pearson Coeff. $= -0.992$; $p << 0.001$; $n = 15$).

The $pH_{(tot)}$ was measured spectrophotometrically twice per week (Hitachi U-2900 dual-beam) using the pH sensitive indicator dye m-cresol purple (Sigma-Aldrich) following SOP (standard operating procedure 6b: Dickson, 2007). Samples of seawater were collected in 20 ml glass scintillation vials (leaving no head space) from all experimental vessels and held in a dark, 12°C water bath for temperature equilibration. Preliminary experiments confirmed no deterioration in measured pH values during the first 8 h after sampling, and pH was always measured within 3–5 h of sample collection. To make each pH measurement, 12 ml of each sample was slowly pipetted into two quartz cuvettes with a 5 cm path length (a modification of the 10 cm path length in SOP 6b). The cuvettes were sealed with a Teflon cover, and held at 12°C in the temperature-controlled chamber of the spectrophotometer. M-cresol purple (10 μl) was added to the sample cuvette, while the second cuvette

Table 1. Mean carbonate chemistry during the 5 weeks incubation.

Treatment (pCO_2)	A_T (µmol kg^{-1})	N (µmol kg^{-1})	P (µmol kg^{-1})	Si (µmol kg^{-1})	C_T (µmol kg^{-1})	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	CO_2 (µmol kg^{-1})	pCO_2-calculated (µatm)	$pH_{(Tot)}$ calculated	Ω_{Ar}	Ω_{Ca}
Control	2316.74 (10.26)	11.20 (0.74)	2.25 (0.96)	6.98 (1.06)	1745.55 (733.73)	2033.33 (11.68)	113.05 (8.56)	23.51 (2.87)	584.3 (71.5)	7.90 (0.04)	1.72 (0.13)	2.69 (0.20)
Mid	2317.31 (7.49)	9.84 (0.23)	1.31 (0.39)	6.26 (0.59)	2272.17 (23.08)	2159.86 (28.12)	62.82 (13.37)	49.49 (8.62)	1232.3 (216.3)	7.61 (0.09)	0.96 (0.20)	1.50 (0.32)
High	2315.43 (6.90)	9.90 (1.98)	1.42 (0.67)	6.28 (0.74)	2330.60 (6.06)	2213.10 (3.64)	40.56 (3.04)	79.95 (7.03)	1912.8 (173.9)	7.42 (0.04)	0.62 (0.05)	0.97 (0.07)

Total alkalinity (A_T), total inorganic carbon (C_T), phosphate and silicate concentrations were collected from one of the replicate tanks every 7–10 d ($n = 3$) and at the beginning and end of the experiment in the other two replicate tanks. Inorganic carbon species, pCO_2, $pH_{(Tot)}$ and the saturation state of aragonite (Ω_{ar}) and calcite (Ω_{ca}) were calculated using CO2sys v2.1. Numbers in parentheses are *SD*.

served as a reference. Absorbance was measured at 578 nm (A1), 434 nm (A2), and 730 nm (background). The sample cuvette was inverted three times and the absorbance was re-measured at the three wavelengths. We used equations in section 8.3 of SOP 6b to correct A1/A2 for the addition of dye. The pK2 and final pH value was determined from Liu *et al.* (2011, equation 18).

Carbonate chemistry was determined from total dissolved inorganic carbon (C_T), total alkalinity (A_T), temperature, salinity, and nutrients (phosphate, silicate, and nitrate). Water was taken from all replicate tanks during the first and third weeks and at the end of the experiment and stored in a 1 l borosilicate flask with a ground glass stopper. The samples were poisoned with a saturated mercuric chloride solution (Riebesell *et al.*, 2010) and stored in the dark at 8°C until analysis. Dissolved inorganic carbon (C_T) was analysed by coulometric titration (Dickson, 2007) using a CM5015 coulometer (UIC Inc., USA) connected to a VINDTA 043 (Marianda, Germany) after acidification with 8.5% phosphoric acid. A_T was analysed by potentiometric titration (Dickson, 2007) in an open cell with 0.1 M HCl using a VINDTA 042 (Marianda, Germany). Certified reference material provided by Andrew Dickson (Scripps Institution of Oceanography, San Diego, USA) was used to calibrate C_T and A_T measurements. An additional sample (20 ml) was collected, passed through a sterile 0.2 µm cellulous acetate syringe membrane filter (VWR-USA), and stored in HDPE bottles with HDPE caps with 50 µl of chloroform added. These samples were analysed for silica, phosphorus, and nitrogen. Carbonate chemistry parameters including $pH_{(T)}$ were calculated using CO2SYS2.1 (Lewis *et al.*, 1998) with the standard set of carbonate system equations and constants of Mehrbach *et al.* (1973) after applying the refit of Dickson and Millero (1987).

Phytoplankton culture and food distribution

The copepods were fed an algal mixture consisting of the cryptophyte, *Rhodomonas baltica* (6–8 µm equivalent spherical diameter, ESD), the prymnesiophyte, *Isochrysis galbana* (4–5 µm ESD), and the diatoms, *Skeletonema costatum* (6–7 µm ESD) and *Chaetoceros mulleri* (4–6 µm ESD). The algae were cultured at 22°C in the phytoplankton culture facility at the Austevoll Research Station. Unialgal batch cultures were incubated in Superba^{TM} NPK 14-4-21 in 100 l cylindrical plastic bags bubbled with CO_2-mixed air under a 24 h light cycle at average light intensities of 100 µE m^{-2} s^{-1}. The batch cultures were harvested in the exponential growth phase. Daily subsamples from the batch cultures were counted and sized using a Z2 Beckman Coulter Counter. Carbon content per cell (*R. baltica*: 18.1 pg cell^{-1}; *I. galbana*: 8.9 pg cell^{-1}; *S. costatum*: 7.9 pg cell^{-1}; *C. mulleri*: 8.1 pg cell^{-1}) was determined from existing carbon volume conversion equations (Strathmann, 1967; Menden-Deuer and Lessard, 2000). The volume of stock culture of each species needed to provide a 50:20:15:15 carbon ratio of *R. baltica*, *I. galbana*, *S. costatum*, and *C. mulleri*, respectively, to the control and treatment tanks was determined periodically. The food mixture was distributed from a 5 l reservoir to each tank using a peristaltic pump to maintain a nominal concentration of 600 µg C l^{-1}, taking into account the average water exchange rate of 10 l h^{-1}. Typical hourly dosage volumes (50–120 ml) of phytoplankton stock solution, with lower pCO_2, were less than 1% of tank volume and did not alter treatment or control levels. Phytoplankton cell concentrations in each tank were monitored daily by counting subsamples using a Coulter Counter, following the methods described in Kim and Menden-Deuer (2013).

Initiation of the experiment

Calanus finmarchicus adult females used to inoculate the control and treatment tanks with eggs were collected in Bjørn afjord using either a light trap or plankton net. A light trap (500 μm mesh BellaMare) was deployed for ~10 h at night from the dock at the research station (water depth: 40 m) at a depth of 20–30 m. Alternatively, copepods were collected using a 0.5 m diameter plankton net towed obliquely at low speed from ~300 m to the surface in the adjacent fjord (water depth: 500 m). The animals were transported to a cold room (12°C) in the laboratory where females were sorted. Females were maintained in large egg separators in 15 l of ambient control seawater and fed the stock algal culture at a concentration sufficient to support maximum feeding rates (i.e. >600 μg C l^{-1}). The containers were routinely monitored for egg production rate and hatching success until the start of the experiment.

The experiment was designed to ensure that all the tanks received eggs from the same population of females and had staggered start dates to allow the team of 2–4 people sufficient time to sample and analyse each tank over the duration of the experiment. Females were evenly divided among four egg separators and immersed in an experimental tank. The egg separators (6 l) were constructed from 25 cm diameter PVC cylinders with a 500 μm nitex mesh bottom that retained females but allowed eggs to pass through. The females were fed using the food distribution system described above. After the females had released eggs for 24–26 h, the cylinders were transferred to the next experimental tank. Tanks were transitioned to the appropriate pCO_2 treatment source immediately after inoculation. The total number of eggs in each tank varied between 13 000 and 55 000 (Table 2). Inoculations of replicate treatment tanks were staggered in a semi-random order. The A and B replicates were used for routine sampling of copepods for the measurements described below. One of the treatment replicates (High A: Table 2) was short lived and had to be restarted (High A, restart). A third replicate (Tank C) was also initiated as a backup for each treatment. Because of the late restart of High A, which did not complete the full developmental cycle by the termination of the experiment, samples of females were taken from High C to supplement measurements of mass for this treatment.

Biological measurements

Individual *C. finmarchicus* (average number: 37) were sampled daily from each tank to assess developmental progress. Age zero for each tank was set at 13 h, the midpoint after the start of inoculation with

Table 2. Daily measurements and tank inoculations.

pH treatment Replicate tank	Inoculation start date *N* (d)	Female number Duration (h)	Egg number EPR	Food (μgC $^{-1}$)	Temp (°C)	Salinity (PSU)	pH (NBS)
Control	1 May 2013	523	13 000	675	12.65	35.11	7.94
A	28	26	24	± 97	± 0.04	± 0.01	± 0.006
Control	8 May 2013	847	43 000	479	12.75	35.12	7.93
B	27	26	47	± 62	± 0.04	± 0.01	± 0.005
Control	4 May 2013	523	31 000	397	12.74	35.13	7.94
C	31	42	34	± 51	± 0.04	± 0.01	± 0.003
Mid	3 May 2013	523	17 000	685	12.73	35.11	7.63
A	33	26	31	± 94	± 0.04	± 0.01	± 0.004
Mid	6 May 2013	847	37 000	443	12.77	35.13	7.62
B	30	26	41	± 52	± 0.04	± 0.01	± 0.004
Mid	9 May 2013	820	44 000	506	12.79	35.13	7.62
C	28	26	50	± 70	± 0.04	± 0.01	± 0.005
High	2 May 2013	523	16 000	573	12.49	35.1	7.49
A	8	26	27	± 155	± 0.04	± 0.00	± 0.015
High	7 May 2013	847	40 000	658	12.8	35.14	7.5
B	22	26	44	± 126	± 0.03	± 0.02	± 0.007
High	11 May 2013	810	48 000	493	12.73	35.13	7.49
C	29	26	55	± 61	± 0.03	± 0.01	± 0.004
High	15 May 2013	800	55 000	782	12.75	35.13	7.51
A, restart	26	26	64	± 136	± 0.03	± 0.01	± 0.005
Treatment (pCO_2)	Replicate	pH (NBS) Electrode	pH (Tot) Spec				
Control	1	7.94 (0.03)	7.918 (0.012)				
	2	7.94 (0.03)	7.925 (0.055)				
	3	7.95 (0.02)	7.918 (0.012)				
Mid	1	7.63 (0.03)	7.618 (0.012)				
	2	7.64 (0.06)	7.611 (0.011)				
	3	7.63 (0.03)	7.621 (0.015)				
High	1	7.50 (0.04)	7.530 (0.067)				
	2	7.51 (0.08)	7.507 (0.073)				
	3	7.50 (0.03)	7.503 (0.038)				

Experimental tank inoculations were staggered in a random order. Number of eggs starting in each tank estimated from the number of females, the inoculation duration and the estimated egg production rate (EPR: eggs female^{-1} d^{-1}) from measurements made near the start, middle, and end of the inoculation period. The daily sampling data begins on the third day after inoculation and continues to the conclusion of that tank (*N*: total duration of sampling period). Temperature, salinity, pH (calibrated electrode readings), and cell counts were measured daily. Food concentration was determined by multiplying cell counts and pg C per algal cell. Mean and standard errors are provided.

Temperature (mean ± SD; $n = 43$), salinity (mean ± SD; $n = 43$), and pH (calibrated electrode; mean ± SD; $n = 43$) values for each treatment during the 5-week incubation. pH (mean ± SD; $n = 10$) was also measured spectrophotometrically twice a week.

females which lasted for 26 h. Animals were subsampled with a large bore pipette immersed in different locations in the tank to obtain a random sample. Each sample was examined (staged and photographed) under a Leica MS5 dissecting scope fitted with a Planapo ×1.0 magnifier and an Olympus DP70 digital camera. The photographs were analysed for body length (nauplius stages) and prosome length (copepodid stages) using ImageJ software (NIH, USA). The area of the lipid oil sac in stage C1–C5 was measured using ImageJ software, and the lipid (wax ester) weight calculated directly from the oil sac area using the perimeter line method (Vogedes *et al.*, 2010).

Additional specimens of the developing life stages were removed from the experimental tanks every 1–3 d (over a 7- week period) to measure dry weight and C and N mass, using methods similar to those reported in Campbell *et al.* (2001). Dry weights of eggs and stages N1–C4 were measured by pipetting a known number of single stage animals onto a pre-combusted 25 mm diameter glass fibre filter mounted on a filter holder and connected to a vacuum pump used to gently suction off seawater. The filter was rinsed with a standard amount of distilled water (3 ml). Controls were routinely run to account for loss in filter weight by this process, presumably from loose fibres that were suctioned off with the water. The preweighed filters were dried in an oven at 60°C for 24–48 h, reweighed to the nearest 1 μg on a Mettler-Toledo UMX2 microbalance, then folded into tin boats for C:H:N analysis. Some of the stage C4, and all the stage C5 and adult animals, were rinsed then placed individually into preweighed tin boats, which were then dried in the oven and re-weighed. The samples were taken back to the Darling Marine Center (Walpole, ME, USA) for measurement of C and N content. Those samples were combusted with a Perkin Elmer 2400 Series II CHNS/O analyser equipped with a thermal conductivity detector using ultra high purity helium as a carrier gas. While the dry weight and carbon mass of individual C5 and early adult stages were well above instrument levels of sensitivity, the N mass of individual C5 and early-stage adults was near the limit of sensitivity of the C:H:N analyser. Individual N masses that were <1 SD above the N blank were discarded as unreliable, resulting in the loss of 32 observations.

Ingestion rate experiments were performed using C5 stage *C. finmarchicus* reared in the experimental tanks. The experiments were run for 24 h in the dark at 13°C, with the animals feeding on the cryptophyte, *Rhodomonas baltica* (Equivalent Spherical Diameter: 6.0 μm) with a measured concentration of 2×10^4 cells ml^{-1} (760 μg C l^{-1}). Copepods from the three pCO_2 treatment levels were handpicked and placed into 2 l flasks at a concentration of four copepods per l. Algal concentrations at the start of the experiment were measured in triplicate using a Beckman Z2 Coulter Counter which required a total of 3 ml of sample (0.15% of total volume). Removed fluid was replaced with the same quantity of fluid at approximately the same algal concentration to ensure that no air bubbles were present in the feeding container. The combination of jar size and the number of copepods used was chosen such that changes in phytoplankton cell numbers due to grazing would be detectable (>5% decrease) yet would not deplete cell concentrations by >25%. Three control flasks (containing algae but no copepods) and three experimental replicates were used for each treatment. All experimental and control flasks were gently turned on a plankton wheel at 0.5 rpm to maintain algae in suspension. After 24 h, each jar was removed from the plankton wheel, and a 1 ml subsample (in triplicate) was withdrawn with a pipette for immediate cell counts. Initial and final cell concentrations were measured in each flask, such that each replicate provided an independent estimate of grazing or phytoplankton growth rate. Contents were then poured through a 100 μm Nitex screen to recover the animals. Copepods were counted and their number, stage, and viability verified. Only live animals were used in the grazing rate calculations. Ingestion rates were calculated based on the equations in Frost (1972).

Oxygen consumption rates (*OCR*s) were measured using animals collected from each replicate tank and sorted for stage and sex (adult) under a binocular microscope. The animals were transferred to the experimental chambers (4.8 ml) filled with seawater (containing no air spaces) from their individual tanks. Experimental chambers were sealed with a ground glass top equipped with a small hole (0.4 mm) to accommodate the oxygen sensitive microelectrode. Between 10 and 30 individuals were used to measure the respiration rates of stages N3 and N6 and 1–5 individuals for adults. Dissolved oxygen concentrations were measured with a Clark-type oxygen microelectrode (Unisense; Aarhus, Denmark). The linear response of each electrode was calibrated with 0.2 μm filtered seawater bubbled for a minimum of 1 h to set the 100% dissolved oxygen calibration point. The anoxic calibration point was determined by placing seawater into a silicone tube that was immersed in a solution of 0.1 M sodium ascorbate and 0.1 M sodium hydroxide for over 4 h. All oxygen measurements were made at 13°C ($\pm$0.01°C) in a ThermoScientific water bath (Model A10B with thermostat SC100). Oxygen concentrations within the chambers, measured every 2 s for up to 1.5 h, never decreased by >20% below saturation. Control chambers without animals were used to monitor oxygen changes due to microbial/algal respiration. Oxygen consumption was computed as the difference between the beginning and end of the incubation, corrected for changes in the control bottles. Data were normalized per unit dry weight obtained from both direct measurements and compared with literature data (Cohen and Lough, 1981).

Data analysis

ANOVA was used to test for differences in experimental conditions among the tanks, and to test for differences among the control and two pCO_2 treatments in the biological variables. When the assumption of normality was not met, non-parametric tests were applied, as specified below. Except where noted, MATLAB statistical software was used to conduct the analyses.

To estimate instantaneous growth rate (g), we applied an exponential growth model ($W_t = W_0e^{gt}$), where W is mass (in terms of dry weight, carbon, or nitrogen), g is growth rate (d^{-1}) and t is age (d). Since the same females were used to inoculate each set of replicate tanks, and egg mass is assumed to be invariant (Runge, 1984), we used a common initial mass for stage N3 (W_0), using values for egg dry weight, C and N mass from Campbell *et al.* (2001) and assuming no weight loss between during the non-feeding stages N1 and N2. Before fitting the data to the model, measurements of individual copepods were averaged according to stage, sampling day, treatment and replicate tank. The mean measurements were more consistent across stages since observations of younger stages were based on up to 100 animals. Exponential growth between stage N3 and early-stage females was assumed. After 32 d, the mass of females is assumed to be constant as energy in females is shunted to egg production (Myers and Runge, 1983). Growth equations were fit to data using the MATLAB fit function.

The effect of pCO_2 treatment on growth rate (stages N6 to early adult) was investigated using ANCOVA, after preliminary exploration to detect outliers and heterogeneity of variance (Zuur *et al.*, 2009). Mass was ln-transformed since it increases exponentially with age and ANCOVA assumes linear relationships. Age was set as the continuous explanatory variable and pCO_2 treatment as the co-factor. Residual plots for the model were inspected and revealed no violation of homoscedasticity. This analysis was conducted in the "R" statistical and programming environment.

Results

Conditions in the control and experimental tanks are summarized in Tables 1 and 2. Calculated CO_2 values were 584 (71.5 SD) μatm, 1232 (216.3) μatm, and 1912 (173.9) μatm for ambient mid and high pCO_2 treatments. Spectrophotometrically measured $pH_{(T)}$ values were 7.920(0.004 SD), 7.617 (0.005 SD), and 7.513 (0.014 SD) for the ambient, mid and high pCO2 treatments, respectively. These values agreed well with the calculated (CO2SYS2.1) pH values for the ambient and mid treatments. For the highest pCO_2 treatment, calculated values underestimated measured $pH_{(T)}$ by 0.09 pH units. The pH within individual tanks remained stable over time and was consistent between replicate tanks. There were no significant differences in daily $pH_{(NBS)}$ among replicates within each treatment (one-way ANOVA, Control: d.f. = 85, $F = 2.45$, $p = 0.09$; Mid: d.f. = 90, $F = 0.3$, $p = 0.74$; High: d.f. = 84, $F = 1.93$, $p = 0.13$). Daily $pH_{(NBS)}$ values (SE < 0.01) were 7.94; 7.62 and 7.50 for ambient, mid and high pCO_2 treatments, respectively (Table 2). The seawater temperature in control and treatment tanks increased throughout the experiment, likely reflecting warming of ambient seawater at the intake, resulting in a net 0.5°C increase across the experiment. The short-lived treatment tank (High A: Table 2) had the lowest mean temperature (12.49°C). Excluding this tank, the overall mean temperature was 12.7°C, with no significant differences among tanks (one-way ANOVA: d.f. = 253, $F = 1.17$, $p = 0.32$). There was no observable change in salinity (overall mean: 35.12) over the course of the experiment.

The overall mean food concentration for the treatment tanks was 564 μg C l^{-1} (Table 2). However, the variance of food concentration in each tank was higher in the first set of replicates (Bartlett test of equal variance; $T = 48.9$; d.f. = 9; $p < 0.001$) which was a consequence of refinements to improve food distribution as the experiment progressed. Food levels were above the critical concentration for *Calanus* species (Frost, 1972; Runge, 1985; Hirche *et al.*, 1997).

Overview of biological measurements

We were able to successfully rear *C. finmarchicus* adults from eggs spawned by wild females at the control (584 μatm), mid-level (1232 μatm), and high-level (1912 μatm) pCO_2 concentrations. In all treatments, the nauplius and copepodid stages appeared healthy and exhibited normal swimming behaviours and evasion responses to our pipettes during sorting.

While we did not quantitatively measure survival rates, we noted large numbers of developing stages in all nine treatment tanks. The one high pCO_2 replicate (High A: Table 2) that suffered high mortality between stage N3–C1 was re-inoculated and had developed successfully to C1 at the time when the entire experiment was terminated. The reason for the crash of this one tank is not known, but there is no evidence that it was due to the pCO_2 treatment. Data from the samples collected from this tank are included in the figures and analyses below.

Development time

Copepod stages were enumerated and summed across replicate tanks on each age day for which there were samples. Development times at control and treatment levels were similar whether calculated as cumulative percentage (Campbell *et al.*, 2001) or weighted stage value (Figures 1 and 2). There was an initial delay of 1–2 d in transition from stages C4 to C5 in the high pCO_2 treatment. However, development at the high pCO_2 treatment returned to the same trajectory as the other treatments within several days and moulting to the adult stage occurred at the same time in all tanks. A Wilcoxon rank-sum test confirmed that there were no significant differences in development time among pCO_2 levels (Control vs. High: $Z = 0.07$, $p = 0.95$; Control vs. Mid: $Z = 0.38$, $p = 0.70$; Mid vs. High: $Z = 0.48$, $p = 0.63$).

Prosome length

There was a small but discernible pCO_2 treatment-related difference in prosome length with respect to age across all stages (ANCOVA: d.f. = 3398, $F = 29$; $p < 0.005$; nauplius $n = 1421$, copepodid stages $n = 1899$, adult females $n = 84$), although there was no consistent trend (Figure 3). The mid-pCO_2 treatment had the largest adjusted (age effect removed) overall mean prosome length 1.035 mm, significantly greater, by ~5%, than the adjusted mean prosome length of both the control (0.989 mm) and high pCO_2 (0.974 mm) treatments, which were not statistically different. Removal of females and nauplii from the analysis did not change this result; the mid pCO_2 adjusted mean prosome length (1.480 mm) was still significantly larger (ANCOVA, d.f. = 2652, $F = 48$, $p < 0.005$) than the control (1.442 mm) and high treatment lengths (1.388 mm). However, the mean prosome length of females in the ambient control (2.568 mm) is significantly larger (ANCOVA, d.f. = 80, $F = 11.1$, $p < 0.005$), by ~6 and 9%, respectively, than the mid (2.431 mm) and high treatment females (2.354 mm). Over the entire dataset, an analysis of covariance attributes <0.2% of the variance to treatment, with the rest of the non-residual variance attributed to age.

Growth rates: dry, carbon, and nitrogen mass

Across all treatments, the dry weight, nitrogen, and carbon mass of *C. finmarchicus* increased exponentially throughout development (Figure 4). Growth rates are more accurately estimated from mass and age in days, regardless of the stage structure on any given day (Figure 5). Fitted growth estimates (to mass and age in days, Figure 5) across the control and elevated pCO_2 treatments ranged between 0.23 and 0.24 d^{-1} in terms of dry weight and C mass, and between 0.21 and 0.22 d^{-1} in terms of N mass (Table 3). ANCOVA analysis showed a significant effect of treatment on overall dry weight, C and N mass (small differences in intercept, notably for the mid pCO_2 treatment) but the interaction term was not significant when related to age. In other words, the rate at which mass increased over time (i.e. growth) was not significantly different between control and treatments (Table 4).

The C:N ratio increased from an overall mean of 4.0 at Stage C1 to 7.9 at Stage C5, reflecting accumulation of lipid mass during copepodid growth. There was no consistent difference in C:N ratio across stages and treatments.

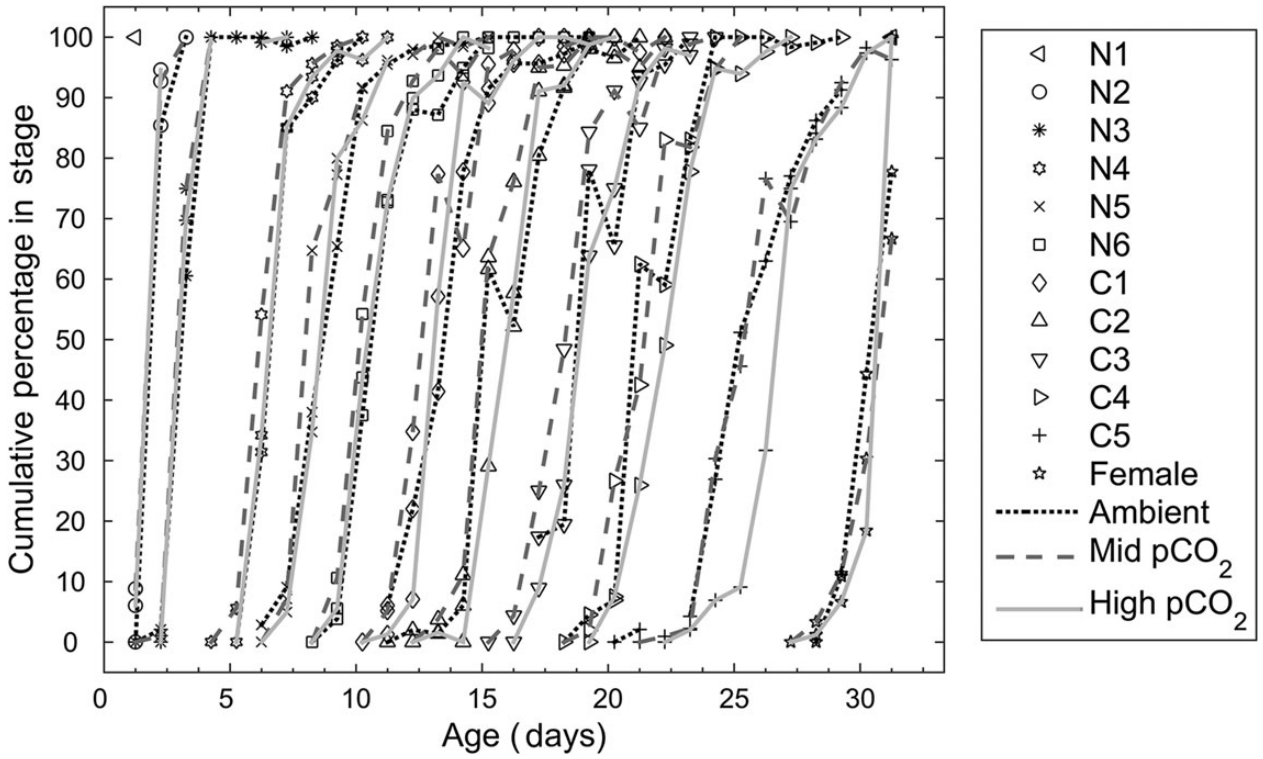

Figure 1. *Calanus finmarchicus*. Stage progression showing development time. Cumulative percentage of copepods (the percentage greater than or equal to a given stage at each sample time) for both replicates of each treatment (ambient mid CO_2 and high CO_2) as shown.

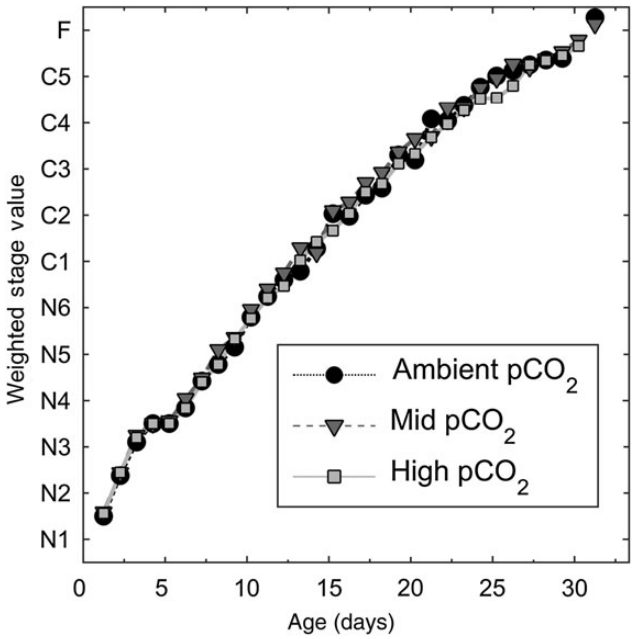

Figure 2. *Calanus finmarchicus* Stage progression in each OA treatment shown by weighted stage value. The weighted stage value determined at each sampling date is equal to the sum of each stage fraction multiplied by its weight (N1 = 1, N2 = 2, N3 = 3 . . . C1 = 7, C2 = 8, C3 = 9 . . . C6 = 12).

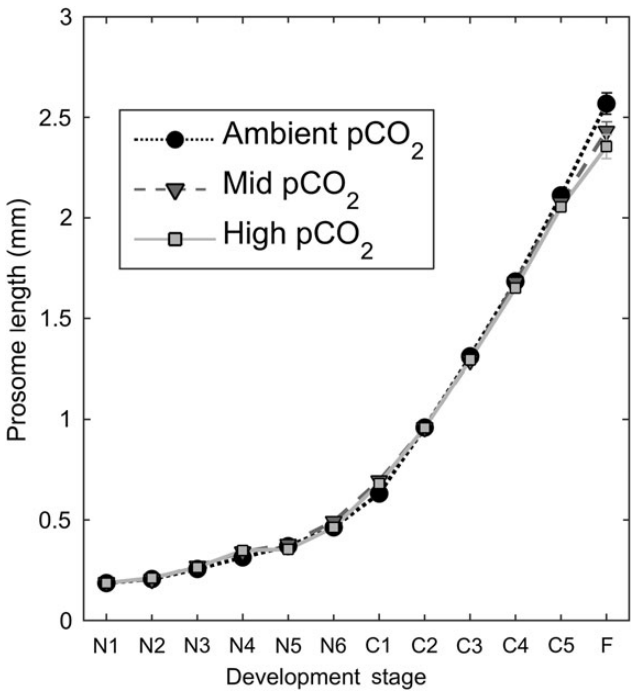

Figure 3. *Calanus finmarchicus* mean prosome length and 95% confidence intervals at each stage in each treatment.

Lipid accumulation

Lipid mass was estimated in 107 mature stage C5 copepods (within 3 d of moulting to adult) from control and higher pCO_2 tanks. The mean lipid/dry mass ratio varied between 26 and 31% and was not significantly different (one-way ANOVA (F (2, 104) = 1.2, $p = 0.305$) among treatments (Figure 6).

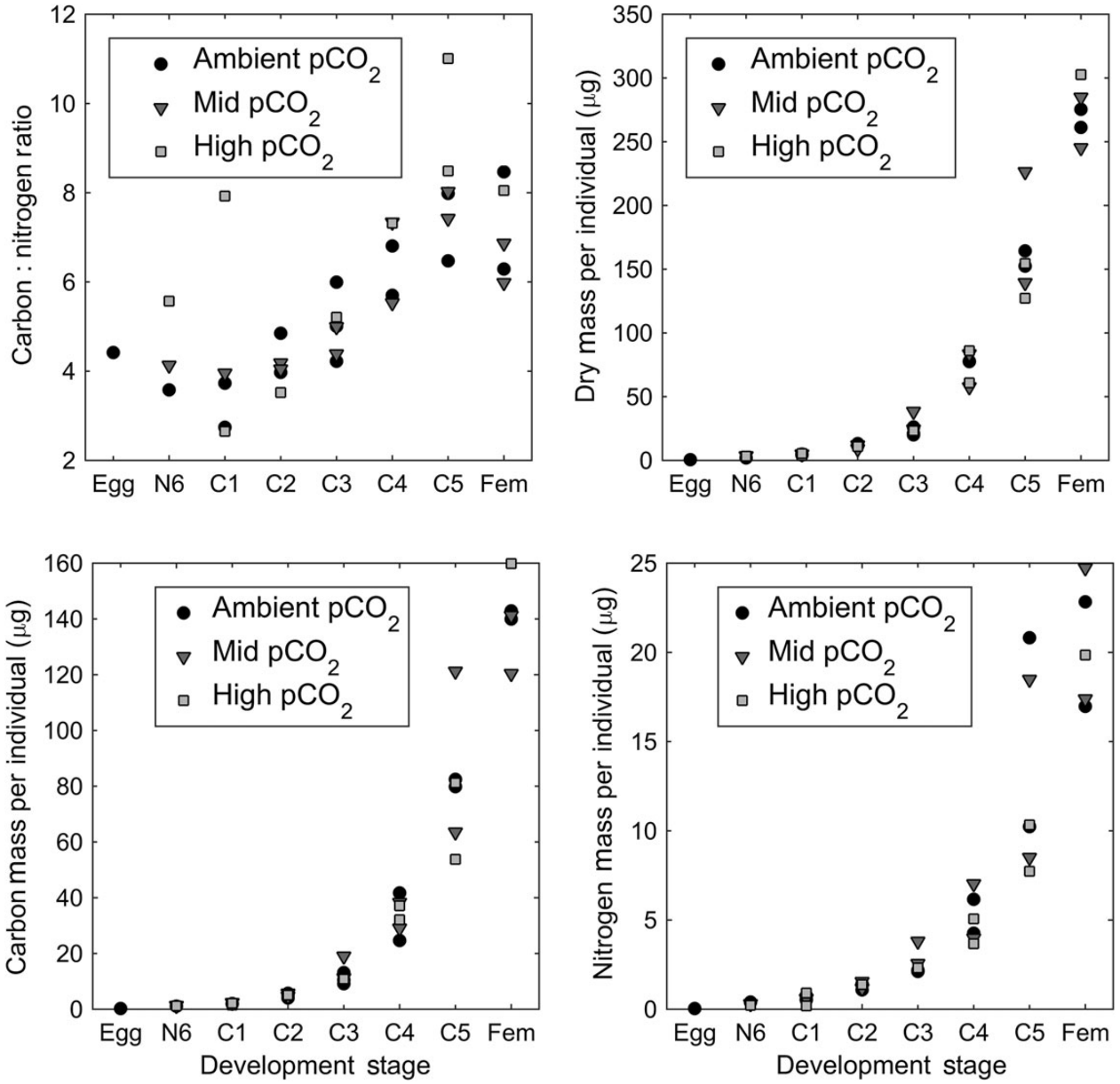

Figure 4. *Calanus finmarchicus*. Dry, carbon, and nitrogen mass (μg) as well as carbon to nitrogen ratio at each stage and treatment: ambient control (circles), mid CO_2 (triangles), and high CO_2 (squares). All replicates are shown. Mass of adult females was measured in only 1 replicate tank for the high CO_2 treatment; the experiment was terminated before individuals from the other high treatment tanks attained adult stage.

Ingestion rates

There were no significant differences in the ingestion rates of *C. finmarchicus* stage C5 reared at different pCO_2 levels (Figure 7: one-way ANOVA; (F (2, 26) = 0.51; p = 0.61). Animals consumed on average 2.2 × 10^5 cells copepod^{-1} d^{-1} (8.37 μg C d^{-1}) with average clearance rates of ~11.0 ml copepod^{-1} d^{-1} at cell concentrations of 2.0 × 10^4 cells ml^{-1}. Total daily ingestion rates were ~10.0% of the average body carbon (Figure 4) for stage C5. This is consistent with the maximum reported rate for adult females of this species (Mauchline, 1998).

Oxygen consumption rates

OCR increased as a function of copepod stage in all pCO_2 treatments. There was no significant difference in *OCR* between the pCO_2 treatments (ANOVA: $p > 0.5$ for N3, N6, C5, Adults). The average *OCR* for adult females at 13°C was 44 nmol O_2 ind^{-1} h^{-1}. The data were fit to a modified exponential curve as a function of stage, $OCR = \exp(6.55(S - -0.69))$, where S = the numerical equivalent for each stage of the copepod (d.f. = 13, F = 221.3, $p < 0.0001$: Figure 8). As a function of dry wt. (DW), *OCR* increased linearly, as $OCR = 0.10 + 0.15 \times DW$, $F(2,13) = 259.4$, $p = <0.001$: Figure 9). When normalized to the copepod's dry weight, the *OCR* decreased linearly with stage, indicating that N6 stage animals consumed 400% more oxygen per unit dry weight than adult females (Figure 9).

Discussion

Effect of pCO_2 on *Calanus finmarchicus* vital rates

Oceanic pCO_2 concentrations predicted to occur during the next century did not directly affect *C. finmarchicus* development rate, growth rate, respiration rates, and feeding rates. Further, our

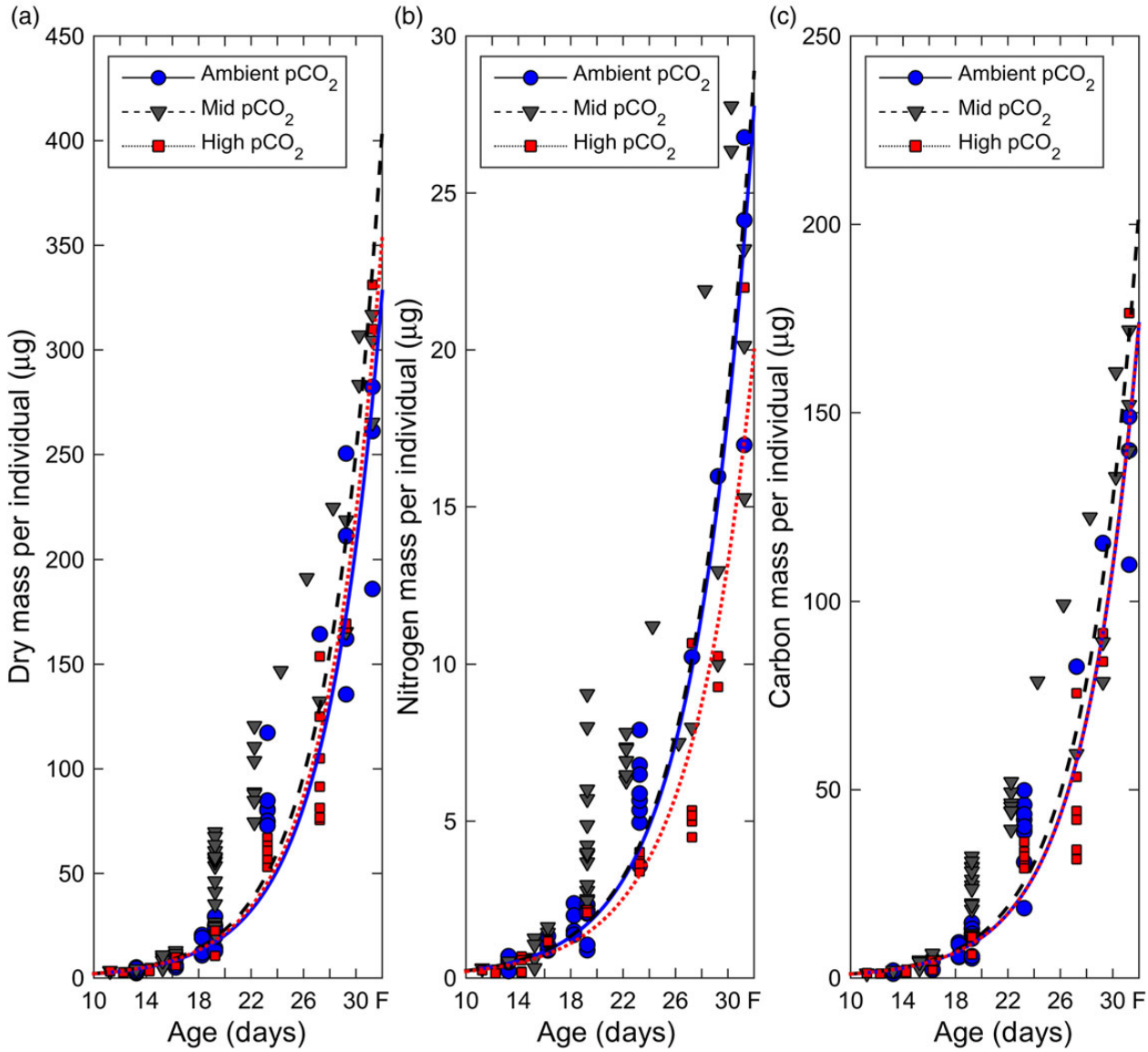

Figure 5. *Calanus finmarchicus*. Increase in mass as a function of time (days) at the ambient control and two treatment levels. (a) Dry weight; (b) nitrogen mass, and (c) carbon mass. Females older than 32 d were considered mature, ages for those observations are set to day 32 in the plots and model fit. Curves represent best overall fit to the growth model ($W_t = W_0 e^{gt}$). Growth rates (g) for the model are indicated. R^2: dry weights, 0.85; nitrogen, 0.79; carbon, 0.86.

qualitative observations did not reveal any indication of differences in survivorship or behaviour. Our analysis did detect a small but significant treatment difference in prosome length and body mass, notably a somewhat *larger* body size in the mid-pCO_2 treatment (i.e. a putatively beneficial effect). This was driven mainly by the higher mass and length of stages C3–C5 in replicate tank Mid A (Table 2). A more precise method for estimating mass at age, perhaps by measuring mass at the time of moult, might resolve whether there is indeed a small and perhaps beneficial treatment effect on body size at age.

Calanus finmarchicus is tolerant of elevated pCO_2

The evidence in support of tolerance of *C. finmarchicus* for predicted future pCO_2 levels is extensive and consistent (Table 5). Experiments have been conducted on all life stages originating from both wild (this study) and cultured females (Pedersen *et al.*, 2014a), including possible carry over effects from an F_1 generation through to the hatching success of eggs from F_2 females (Pedersen *et al.*, 2014a). These studies suggest that parental history (i.e. wild vs. cultured) is not an influential factor and they allay concerns about misrepresenting pCO_2 effects when extrapolating from one life stage (or sex) to another (Cripps *et al.*, 2014). While we did not measure them in this study, previous research is consistent in finding no effect of increased pCO_2 concentrations (<2000 µatm) on egg production rate, fertilization, or egg hatching success (Mayor *et al.*, 2007; Preziosi, 2012; Pedersen *et al.*, 2014a, b). Pedersen *et al.* (2014a) reported a significant decrease in body length and dry weight (by 5–25%), as well as a significant increase (by up to 40%) in respiration rate in stage C5, as treatment pCO_2 increased from ambient to 3482 µatm. However, this interpretation is based on linear regressions across all pCO2 treatment levels. Inspection of these data (Pedersen *et al.*, 2014a: Figure 2) indicates

Table 3. Estimate of instantaneous growth rate, g (d^{-1}), in terms of dry weight (μg), nitrogen mass (μgN), and carbon mass (μgC) at each pCO_2 treatment, assuming exponential growth between egg and early female stages.

	g	CI	*N*	r^2
Dry weight				
Ambient	0.231	0.004	37	0.85
Mid CO2	0.239	0.004	44	0.81
High CO_2	0.234	0.002	34	0.96
Nitrogen				
Ambient	0.218	0.003	34	0.93
Mid CO_2	0.219	0.005	44	0.70
High CO_2	0.207	0.004	30	0.88
Carbon				
Ambient	0.232	0.003	34	0.90
Mid CO_2	0.237	0.004	44	0.83
High CO_2	0.232	0.004	31	0.90

The starting stage N3 mass (W_0) is assumed to be identical in control and treatments. Individual mass measurements of stages C4 and C5 were averaged at each age (d). Model fitted in MATLAB. CI: 95% confidence interval, *N*: number of data points, and r^2 of model fits.

Table 4. Summary of the ANCOVA carried out on copepod mass (DW, dry weight; N, nitrogen; C, carbon) raised under three different pCO_2 treatments.

	D.F.	SS	MS	*F*-value	*p* value
Response variable: ln (DW)					
Age	1	222.82	222.82	1344.55	<0.0001
Treatment	2	8.88	4.441	26.80	<0.0001
Age:treatment	2	0.36	0.181	1.09	0.339
Residuals	109	18.06	0.166		
Response variable: ln (N)					
Age	1	151.19	151.187	749.8287	<0.0001
Treatment	2	10.22	5.111	25.3479	<0.0001
Age:treatment	2	0.29	0.143	0.7085	0.495
Residuals	102	20.57	0.202		
Response variable: ln (C)					
Age	1	209.67	209.67	1141.74	<0.0001
Treatment	2	9.25	4.63	25.19	<0.0001
Age:treatment	2	0.63	0.31	1.71	0.187
Residuals	103	18.91	0.18		

Age is days from inoculation. Multiple R^2: 0.93 (DW); 0.89 (N); 0.92 (C). D.F., degrees of freedom; SS, sum of squares; MS, mean square.

no change in any of these variables at pCO_2 concentrations up to the medium treatment level of 2020 μatm; always, the linear regression is driven by lower values at the highest concentration of 3482 μatm. Therefore, based on the evidence from our experiments and from other studies, we conclude that parameterization of physiological rates and processes determining *C. finmarchicus* vital rates in models of population dynamics of this species do not have to be adjusted to account for direct effects of ocean acidification at pCO_2 concentrations <2000 μatm.

Possible threshold response of *Calanus finmarchicus* to elevated pCO_2

Some studies on the effect of pCO_2 on *C. finmarchicus* indicate that there may be a response at concentrations >2000 μatm. For example, Pedersen *et al.* (2014a) observed significant differences in the egg production rates of females at their highest pCO_2 treatment (3482 μatm), although Mayor *et al.* (2007) found no effect on food-limited egg production rates at 8000 μatm. Both Mayor *et al.* (2007) and Pedersen *et al.* (2013) reported substantial egg mortality at 8000 μatm. Cumulative development time at stage C5 of the parental generation was delayed by 2.5 and 3.8 d at pCO_2 concentrations of 2200 and 3500 μatm, respectively, but only at 3500 μatm in the F_1 generation (Pedersen *et al.*, 2014a). Based upon these results, we suggest that pCO_2 impacts on *C. finmarchicus* vital rates would be best modelled as a step function, with effects occurring only above a threshold of ~3000 μatm, corresponding to pH values <7.2. Importantly, however, these values are far beyond predictions of oceanic pH levels over the next several hundred years. Moreover, this threshold concept does not take into account the possibility for adaptation by the organism that would potentially mitigate the effects of even these high pCO_2 levels.

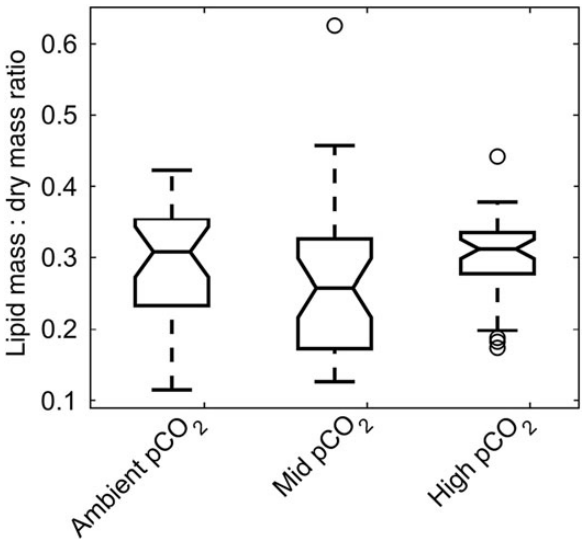

Figure 6. *Calanus finmarchicus*. Lipid mass to dry mass ratio, stage C5. One-way ANOVA: $p = 0.305$; $n = 29, 34, 44$, for ambient, mid, and high CO_2 treatments, respectively.

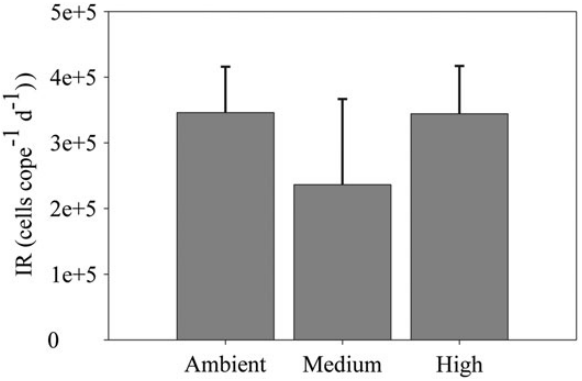

Figure 7. *Calanus finmarchicus*. Ingestion rates of adult females reared in different pCO_2 conditions. One-way ANOVA: $p = 0.095$; $n = 6, 10, 15$, for ambient, mid, and high CO_2 treatments, respectively.

Possible indirect and multiple stressor effects of pCO_2 on *Calanus finmarchicus*

The possibility of indirect effects of pCO_2 on copepods (*sensu* Reum *et al.*, 2015) have received little attention. The type and quality of the food available for *C. finmarchicus* to graze might be altered by pCO_2

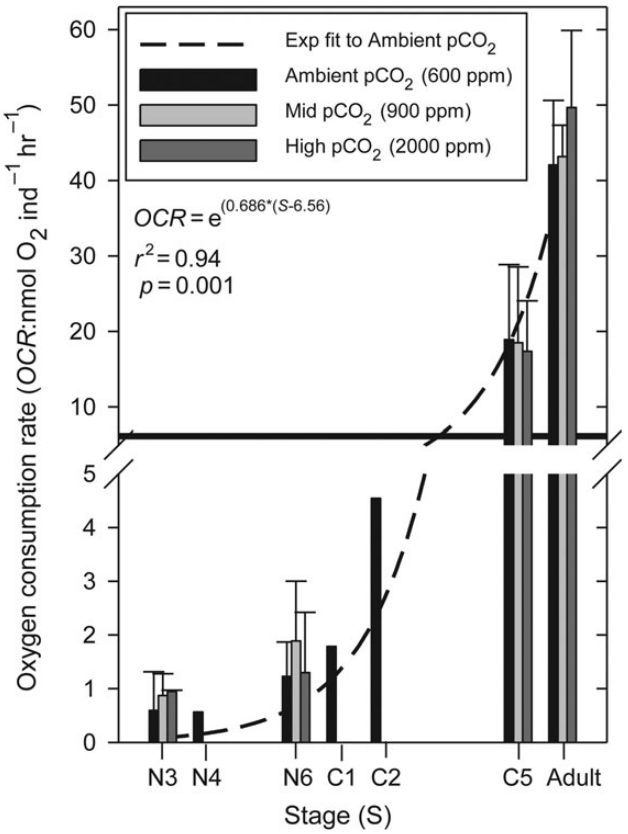

Figure 8. *Calanus finmarchicus*. *OCRs* of adult females reared in different pCO_2 conditions. Measurements at N3, N6, C5, and adult stages were made for all CO_2 treatments. ANOVA at each stage found no significant difference between treatments. *OCR* for N4, C1, and C2 stages were measured only at ambient CO_2 levels. Curve was fit to the pooled data from all measurements.

(Rossoll *et al.*, 2012), affecting the copepod's ability to accumulate or synthesize key proteins, carbohydrates, or fatty acids. While our experiments were conducted at non-limiting food concentrations, food limitation might prevent individuals from obtaining sufficient energy to cover the possible added energetic costs of maintaining intracellular pH levels (Whiteley, 2011). However, two studies (Mayor *et al.*, 2007; Pedersen *et al.*, 2014a) found that egg production rates in food-limited animals were not affected by pCO_2 levels <2020 μatm. Moreover, food was limiting in the growth experiments conducted by Pedersen *et al.* (2014a). Thus, the evidence indicates that, even when food is limiting, growth and development are not affected by pCO_2.

The timing of entry and exit from diapause, and its success, are critical to the modelling of *C. finmarchicus* population dynamics (e.g. Maps *et al.*, 2012). Because pCO_2 concentrations at the depths at which diapause occurs (500–2000 m: Heath *et al.*, 2008) are already at 800–1500 μatm (Hoffman *et al.*, 2013), present adaptation to elevated pCO_2 levels would be expected (Lewis *et al.*, 2013). However, successful diapause at depth is hypothesized to be dependent on fatty acid composition of the wax esters (Pond *et al.*, 2012), and effects of increased surface layer pCO_2 concentrations on fatty acid composition have not been studied.

There is also the possibility of multiple stressor interactions affecting physiological rates of *C. finmarchicus*. For example, an interaction between elevated surface layer temperature and pCO_2 concentrations would have ecological relevance for understanding shifts in distribution at the southern margins of the species' range. However, Preziosi (2012) did not find an effect of elevated temperature (15°C) on hatching success at pH levels >7.4, suggesting that the threshold for a pCO_2 effect may be lower, but still not of concern in the context of predicted increases in either temperature or pCO_2. Nevertheless, further investigation of the possible combined effects of increasing temperature and pCO_2 concentration in surface waters on *C. finmarchicus* growth and development may be useful in the context of modelling the population dynamics under future scenarios at the southern margins of the species distribution.

The response of other copepods to elevated pCO_2

Calanus finmarchicus is currently the most studied species among the Copepoda with respect to the effects of pCO_2. It is, therefore, reasonable to assess how well it represents the responses of other species of *Calanus*, and other copepod species generally. Whiteley (2011) concludes that there is considerable variability in the response of crustaceans to pCO_2, but suggests that species exposed to a broad range of environmental conditions should be better equipped physiologically to tolerate increased pCO_2 and lower pH. As vertical migrators, older life stages of *C. finmarchicus* experience daily change between the ambient pCO_2 levels at the surface and higher pCO_2 levels at depth (up to 250 m: Plourde *et al.*, 2001) and, in addition, stage C5 *C. finmarchicus* overwinter for months in deep water, as noted above. Other *Calanus* species have similar daily and ontogenetic migration behaviours and may, therefore, be more tolerant than smaller copepod species that always reside in surface waters (Lewis *et al.*, 2013). Studies of responses of Arctic species *C. glacialis* and *C. hyperboreus*, as well as the more temperate *C. helgolandicus* (Supplementary Table S1), generally report no effects at pCO_2 concentrations up to 3000 μatm. However, Lewis *et al.* (2013) reported lower survival of nauplius stages believed to be a mixture of *C. glacialis* and *C. hyperboreus* at pCO_2 concentrations as low as 700 μatm. If borne out, this suggests that even among *Calanus* spp. there is species and possibly population specificity in response.

We reviewed what is currently known about the effects of increased pCO_2 across the Copepoda (Supplementary Table S1). Not all life stages or life history characteristics have been investigated within any one species. Nevertheless, significant effects of pCO_2 concentrations <2000 μatm have been reported in only three studies. Effects on egg hatching success and nauplius survival of the estuarine and coastal copepod, *Acartia tonsa*, have been reported at pCO_2 concentrations of 1000 μatm (Cripps *et al.*, 2014). Survival of adults and nauplii of the small but common cyclopoid copepod, *Oithona similis*, as well as the nauplius stages of *C. glacialis* (and possibly, *C. hyberboreus*, discussed above) were negatively affected at pCO_2 concentrations as low as 700 μatm (Lewis *et al.*, 2013). Finally, body size and nauplius survival at low pCO_2 concentrations (400–600 μatm) have been reported for the harpacticoid copepod, *Tisbe battagliai* (Fitzer *et al.*, 2012). All these species are either broadly distributed geographically and/or are exposed to a very wide range of environmental conditions. The life stages of *Oithona* and *Tisbe*, and the nauplius stages of *C. glacialis* and *C. hyperboreus*, are relatively small, consistent with the argument that smaller copepods are more sensitive than larger ones. However, many of the other species listed in Supplementary Table S1 are also small, and we have discussed above that no effect of pCO_2 concentrations <2000 μatm have been observed in the

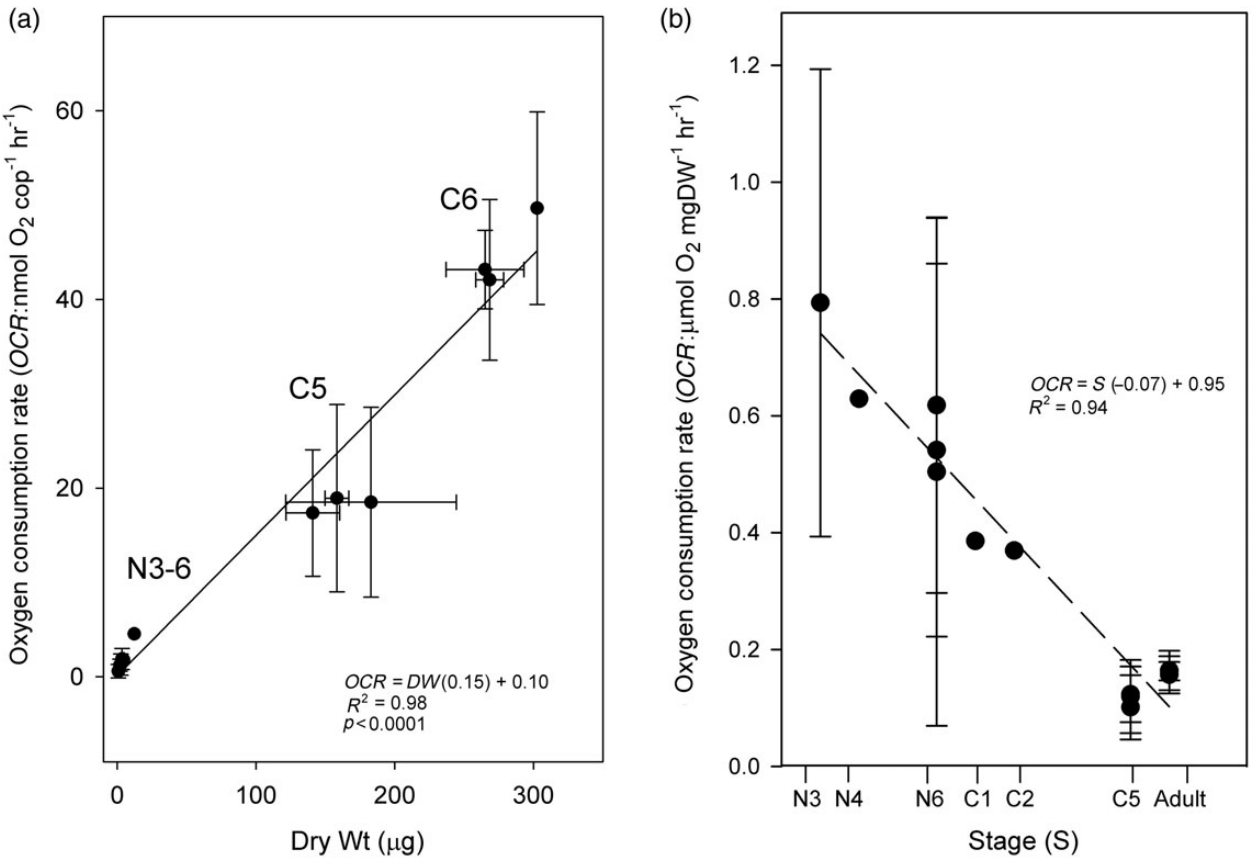

Figure 9. *Calanus finmarchicus*. *OCR*s of individual stages reared in different CO_2 conditions. Measurements at N3, N6, CV, and adult stages were made for all CO_2 treatments. *OCR* for N4, C1, and C2 stages were measured only at ambient CO_2 levels. Dry wts were taken from bulk measurements shown in Fig 4. (a) Relationships between dry weight and *OCR*. (b) *OCR* normalized dry weight as a function of developmental stage.

Table 5. Summary of experimental results investigating CO_2 treatment effects on life history traits of *C. finmarchicus*.

Life history trait	Impact of CO_2 < 1900 µatm	Highest CO_2 level (µatm) at which no effect	Comments
Egg production rate	No[1,4]	8000[1]/2020[4]	No effect on food limited F_0 females[1]; Food limited EPR of F_1 females reduced by 70% at 3500 µatm[4]
Egg hatching success	No[1,3,5]	3500[3]	90% decline observed at 8000 µatm[1] 41% decline observed at 8800 µatm, although not significant[3]
Survival, nauplius stages	No[1,2]	4600[3]	Survival to N2/N3 reduced by 75% at 8800 µatm[3]
Survival, copepodid stages	No[2]	3300[2]	Survival to stage C5 reduced by 49% at 7300 µatm; no survival at 9700 µatm[2]
Development rate, nauplius	No[3,4,6]	4600[3]	Development of nauplius stages N1 – N3 reduced at 8800 µatm
Development rate, copepodid	No[3,4,6]	1912[6]	Development to C5 of F_0 delayed by 7% at 2307 µatm Development to C4 of F_1 delayed by 32% at 3482 µatm
Prosome length	No[4,6]	1912[6]/2020[4]	Prosome length of F_1 decreased by ~5% at 3482 µatm[4] (see text)
Stage CV dry mass	No[6]/possible[4]	1912[6]	F_1 Stage C5 dry mass reduced by 20% at 2020 µatm (but see text) and by 36% at 3482 µatm[4]; no change in stage C5 dry mass at 1912 µatm[6]
Stage CV C mass	No[6]	1912[6]	No data for effects on C5 C mass at CO_2 levels >1912 µatm[6]
Stage CV N mass	No[6]	1912[6]	No data for effects on C5 C mass at CO_2 levels >1912 µatm[6]
Lipid accumulation	No[4,6]	1912[6]/2020[4]	Oil sac volume reduced by 42% at 3482 µatm[4]
Feeding rate	No[4,6]	3500[4]	No change in F_1 stage C5 feeding rate at 3482 µatm[4]
Respiration rate	No[6]/possible[4]	1912[6]	F_1 stage C% respiration rates 15% higher at 2020 µatm (but see text) and 30% higher at 3482 µatm[4]
Predator evasion	No[6]	1912[6]	Qualitative observations[6]

References: [1]Mayor *et al.* (2007), [2]Pedersen *et al.* (2013), [3]Pedersen *et al.* (2014a), [4]Pedersen *et al.* (2014b), [5]Preziosi (2012), and [6]this study.

relatively small early life stages of *C. finmarchicus*. Interestingly, two of the three studies that report an effect of pCO_2 were conducted using animals that had been maintained in culture for many generations. Further research is needed to understand the extent to which some species may or may not be affected by relatively low pCO_2 concentrations and why, as well as their capacity to adapt (physiologically, epigenetically, and genetically) to changing conditions.

The tolerance of modern populations of *C. finmarchicus* to increased pCO_2 appears challenged somewhere ~3000 μatm (pH of ~7.3). This appears true for other species of copepods as well. This threshold may be lower if individuals are simultaneously challenged by other environmental stressors. Whiteley (2011) reviews the mechanisms used by crustaceans to buffer the pH of their internal fluids using haemolymph proteins and bicarbonate ions (HCO_3^-). If the same mechanisms are used by copepods, perhaps there is a threshold buffering capacity that is exceeded at very high pCO_2 concentrations. Comparison of the physiology and epigenetic/genetic control of intracellular buffering capacity of *C. finmarchicus* with that of a copepod species exhibiting low tolerance to elevated pCO_2 would provide insight into the basis for this physiological limit.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Acknowledgements

We gratefully acknowledge R. Campbell for guidance on culturing methods, R. Jones for technical assistance during early phases of the study, K. Thornton for assistance with analysis of copepod carbon and nitrogen mass, and I. Altman for comments on growth rate analysis.

Funding

This research was supported by projects from the Institute of Marine Research, Norway (Project #83192-01) and the Fram Centre (Project #13717-06) to HIB. JAR and CRST were additionally supported by the National Science Foundation award OCE-1041081. DMF also received support from NSF award OCE-1220068.

References

Andersen, S., Grefsrud, E. S., and Harboe, T. 2013. Effect of increased pCO_2 level on early shell development in great scallop (*Pecten maximus* Lamarck) larvae. Biogeosciences, 10: 6161–6184.

Baumgartner, M. F., Mayo, C. A., and Kenney, R. D. 2007. Enormous carnivores, microscopic food and a restaurant that's hard to find. *In* The Urban Whale: North Atlantic Right Whales at the Crossroads, pp. 138–171. Ed. by S. D. Kraus, and R. M. Rolland. Harvard University Press, Cambridge, MA.

Caldeira, K., and Wickett, M. E. 2003. Anthropogenic carbon and ocean pH. Nature, 425: 365.

Campbell, R. G., Wagner, M. M., Teegarden, G. J., Boudreau, C. A., and Durbin, E. G. 2001. Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. Marine Ecology Progress Series, 221: 161–183.

Carlotti, F., Giske, J., and Werner, F. 2000. Modeling zooplankton dynamics. *In* ICES Zooplankton Methodology Manual. Ed. by R. P. Harris, P. Wiebe, J. Lenz, M. Huntley, and H. R. Skjoldal. Academic Press, New York.

Cohen, R. E., and Lough, R. G. 1981. Length-weight relationships for several copepods dominant in the Georges Bank-Gulf of Marine area. Journal of Northwest Atlantic Fishery Science, 2: 47–52.

Cripps, G., Lindeque, P., and Flynn, K. J. 2014. Have we been underestimating the effects of ocean acidification in zooplankton? Global Change Biology, 20: 3377–3385.

Curchitser, E. N., Batchelder, H. P., Haidvogel, D. B., Fiechter, J., and Runge, J. 2013. Advances in physical, biological, and coupled ocean models during the US GLOBEC program. Oceanography, 26: 52–67.

Dickson, A. G. 2007. SOP7 Determination of the pH of sea water using the indicator dye m-cresol purple. *In* Guide to Best Practice for Ocean CO2 Measurements. PICES Special Publication. 191 pp.

Dickson, A. G., and Millero, F. J. 1987. A comparison of the equilibrium constants for the dissociation of carbonic-acid in seawater media. Deep-Sea Research Part A-Oceanographic Research Papers, 34: 1733–1743.

Dupont, S., and Pörtner, H.-O. 2013. A snapshot of ocean acidification research. Marine Biology, 160: 1765–1771.

Dupont, S., Dorey, N., and Thorndyke, M. 2010. What can meta-analysis tell us about vulnerability of marine biodiversity to ocean acidification? Estuarine, Coastal and Shelf Science, 89: 182–185.

Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science, 65: 414–432.

Fitzer, S. C., Caldwell, G. S., Close, A. J., Clare, A. S., Upstill-Goddard, R. C., and Bentley, M. G. 2012. Ocean acidification induces multigenerational decline in copepod naupliar production with possible conflict for reproductive resource allocation. Journal of Experimental Marine Biology and Ecology, 418: 30–36.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnology and Oceanography, 17: 805–815.

Garrard, S. L., Hunter, R. C., Frommel, A. Y., Lane, A. C., Phillips, J. C., Cooper, R., Dineshram, R., *et al.* 2013. Biological impacts of ocean acidification: a postgraduate perspective on research priorities. Marine Biology, 160: 1789–1805.

Gaylord, B., Hill, T. M., Sanford, E., Lenz, E. A., Jacobs, L. A., Sato, K. N., Russell, A. D., *et al.* 2011. Functional impacts of ocean acidification in an ecologically critical foundation species. Journal of Experimental Biology, 214: 2586–2594.

Heath, M. R., Rasmussen, J., Ahmed, Y., Allen, J., Anderson, C. I. H., Brierley, A. S., Brown, L., *et al.* 2008. Spatial demography of *Calanus finmarchicus* in the Irminger Sea. Progress in Oceanography, 7: 39–88.

Hirche, H-J., Meyer, U., and Niehoff, B. 1997. Egg production of *Calanus finmarchicus*: effect of temperature, food and season. Marine Biology, 127: 609–620.

Hjollo, S. S., Huse, G., Skogen, M. D., and Melle, W. 2012. Modelling secondary production in the Norwegian Sea with a fully coupled physical/primary production/individual-based Calanus finmarchicus model system. Marine Biology Research, 8: 508–526.

Hoffman, A. F., Peltzer, E. T., and Brewer, P. G. 2013. Kinetic bottlenecks to chemical exchange rates for deep-sea animals – Part 2: carbon dioxide. Biogeosciences, 10: 2409–2425.

Johnson, C. L., Runge, J. A., Curtis, K. A., Durbin, E. G., Hare, J. A., Incze, L. S., Link, J. S., *et al.* 2011. Biodiversity and ecosystem function in the Gulf of Maine: pattern and role of Zooplankton and Pelagic Nekton. PLoS ONE, 6: 1–18.

Kim, H., and Menden-Deuer, S. 2013. Reliability of rapid, semi-automated assessment of plankton abundance, biomass, and growth rate estimates: Coulter Counter versus light microscope measurements. Limnology Oceanography Methods, 11: 382–393.

Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, 13: 1419–1434.

Lewis, C. N., Brown, K. A., Edwards, L. A., Cooper, G., and Findlay, H. S. 2013. Sensitivity to ocean acidification parallels natural pCO_2 gradients experienced by Arctic copepods under winter sea ice.

Proceedings of the National Academy of Sciences of the United States of America, 110: E4960–E4967.

Lewis, E., Wallace, D., and Allison, L. J. 1998. CO2SYS – program developed for CO_2 system calculations. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Liu, X., Patsavas, M. C., and Byrne, R. H. 2011. Purification and characterization of meta-Cresol purple for spectrophotometric seawater pH measurements. Environmental Science & Technology, 45: 4862–4868.

Maps, F., Runge, J. A., Leising, A., Pershing, A. J., Record, N. R., Plourde, S., and Pierson, J. J. 2012. Modelling the timing and duration of dormancy in populations of *Calanus finmarchicus* from the Northwest Atlantic shelf. Journal of Plankton Research, 34: 36–54.

Maps, F., Zakardjian, B., Plourde, S., and Saucier, F. J. 2011. Modeling the interactions between the seasonal and diel migration behaviors of Calanus finmarchicus and the circulation in the Gulf of St. Lawrence (Canada). Journal of Marine Systems, 88: 183–202.

Mauchline, J. 1998. The Biology of Calanoid Copepods. Academic Press, San Diego. 710 pp.

Mayor, D. J., Matthews, C., Cook, K., Zuur, A. F., and Hay, S. 2007. CO_2-induced acidification affects hatching success in *Calanus finmarchicus*. Marine Ecology-Progress Series, 350: 91–97.

Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowic, R. M. 1973. Measurement of apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography, 18: 897–907.

Melle, W., Runge, J., Head, E., Plourde, S., Castellani, C., Licandro, P., Pierson, J., *et al.* 2014. The North Atlantic Ocean as habitat for *Calanus finmarchicus*: Environmental factors and life history traits. Progress in Oceanography, 129: 244–284.

Menden-Deuer, S., and Lessard, E. J. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography, 45: 569–579.

Myers, R. A., and Runge, J. A. 1983. Predictions of seasonal natural mortality rates in a copepod population using life-history theory. Marine Ecology Progress Series, 11: 189–194.

Pedersen, S. A., Hakedal, O. J., Salaberria, I., Tagliati, A., Gustavson, L. M., Jenssen, B. M., Olsen, A. J., *et al.* 2014a. Multigenerational exposure to ocean acidification during food limitation reveals consequences for copepod scope for growth and vital rates. Environmental Science & Technology, 48: 12275–12284.

Pedersen, S. A., Hansen, B. H., Altin, D., and Olsen, A. J. 2013. Medium-term exposure of the North Atlantic copepod *Calanus finmarchicus* (Gunnerus, 1770) to CO_2-acidified seawater: effects on survival and development. Biogeosciences, 10: 7481–7491.

Pedersen, S. A., Vage, V. T., Olsen, A. J., Hammer, K. M., and Altin, D. 2014b. Effects of elevated carbon dioxide (CO_2) concentrations on early developmental Stages of the marine copepod *Calanus finmarchicus Gunnerus* (Copepoda: Calanoidae). Journal of Toxicology and Environmental Health-Part A-Current Issues, 77: 535–549.

Plourde, S., Joly, P., Runge, J. A., Zakardjian, B., and Dodson, J. 2001. Life cycle of *Calanus finmarchicus* in the lower St. Lawrence Estuary: the imprint of circulation and late timing of the spring phytoplankton bloom. Canadian Journal of Fisheries and Aquatic Sciences, 58: 647–658.

Pond, D. W., Tarling, G. A., Ward, P., and Mayor, D. J. 2012. Wax ester composition influences the diapause patterns in the copepod *Calanoides acutus*. Deep-Sea Research Part II-Topical Studies in Oceanography, 59: 93–104.

Preziosi, B. 2012. The effects of ocean acidification and climate change on the reproductive processes of the marine copepod, *Calanus finmarchicus*. MSc thesis, University of Maine, Orono. 41 pp.

Reum, J. C. P., Ferris, B. E., McDonald, P. S., Farrell, D. M., Harvey, C. J., Klinger, T., and Levin, P. S. 2015. Evaluating community impacts of ocean acidification using qualitative network models. Marine Ecology Progress Series, 536: 11–24.

Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P. 2010. Guide to best practices for ocean acidification research and data reporting. Office for Official Publications of the European Union, Luxembourg.

Rossoll, D., Bermudez, R., Hauss, H., Schulz, K. G., Sommer, U., and Winder, M. 2012. Ocean acidification-induced food quality deterioriation constrains trophic transfer. PLoS ONE, 7: e34737.

Runge, J. A. 1984. Egg production of *Calanus pacificus* Brodsky: laboratory observations. Journal of Experimental Marine Biology and Ecology, 74: 53–66.

Runge, J. A. 1985. Relationship of egg production of *Calanus pacificus* Brodsky to seasonal changes in phytoplankton availability in Puget Sound, Washington. Limnology and Oceanography, 30: 382–396.

Runge, J. A., Ji, R., Thompson, C. R. S., Record, N. R., Chen, C., Vandemark, D. C., Salisbury, J. E., *et al.* 2015. Persistence of Calanus finmarchicus in the western Gulf of Maine during recent extreme warming. Journal of Plankton Research, 37: 221–232.

Skjoldal, H. R. 2004. The Norwegian Sea Ecosystem. Tapir, Trondheim.

Speirs, D. C., Gurney, W. S. C., Heath, M. R., Horbelt, W., Wood, S. N., and de Cuevas, B. A. 2006. Ocean-scale modelling of the distribution, abundance, and seasonal dynamics of the copepod *Calanus finmarchicus*. Marine Ecology Progress Series, 313: 173–192.

Strathmann, R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnology and Oceanography, 12: 411–418.

Trenkel, V. M., Huse, G., MacKenzie, B. R., Alvarez, P., Arrizabalaga, H., Castonguay, M., Gani, N., *et al.* 2014. Comparative ecology of widely distributed pelagic fish species in the North Atlantic: implications for modelling climate and fisheries impacts. Progress in Oceanography, 129: 219–243.

Vogedes, D., Varpe, O., Soreide, J. E., Graeve, M., Berge, J., and Falk-Petersen, S. 2010. Lipid sac area as a proxy for individual lipid content of arctic calanoid copepods. Journal of Plankton Research, 32: 1471–1477.

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. Marine Ecology Progress Series, 430: 257–271.

Williams, R., and Conway, D. V. P. 1988. Vertical distribution and seasonal numerical abundance of the Calanidae in oceanic waters to the south-west of the British Isles. Hydrobiologia, 167/168: 259–266.

Wittmann, A. C., and Poertner, H-O. 2013. Sensitivities of extant animal taxa to ocean acidification. Nature Climate Change, 3: 995–1001.

Zuur, A. F., Ieno, E. N., and Elphick, C. S. 2009. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution, 1: 314.

Handling editor: C. Brock Woodson